DeepVision™ VIS/NIR-I/NIR-II/SWIR Small Animal Fluorescence Imaging System

DeepVision™ Imaging Instrument for Novel NIR-I and NIR-II Dyes and Probes

NIRMIDAS BIOTECH

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In Vivo NIR-II/SWIR Fluorescence Imaging

In vivo fluorescence imaging of small animals has been done typically in the NIR-I (800-900 nm) range, suffering from shallow imaging depth and high background due to light scattering and tissue autofluorescence. NIR-II/SWIR imaging is a breakthrough development detecting fluorescence/luminescence in the 1000-1700 nm range to suppress these effects, affording single cell resolution at ~ 3 nm depth and useful feature resolution up to ~ 1 cm depth. Combined with ultra-bright NIR-II probes (molecules, quantum dots and rare-earth nanoparticles licensed from Stanford University) from Nirmidas Biotech and a new camera technology (10X shorter exposure time and lower background compared than older brands/markers), DeepVision™ affords high performance non-invasive imaging of vasculatures, tumors, intact mouse brain, lymphatic vessels/lymph nodes and molecular imaging using antibody conjugated probes. It is a new generation of NIR imaging instrument empowering researchers to interrogate cardiovascular, cancer, brain and immune disease models.

A unique feature of DeepVision™ is its fluorescence imaging mode. By setting a delay time of detection, the emission generated by a 975 nm excitation pulse from rare-earth nanoparticles (lifetime ~ 10 milliseconds) versus the emission from quantum dots (lifetime in micro-second range) can be distinguished despite their overlapping emission wavelengths. Such a scheme can be used for multiplexed molecular imaging in the least scattering 1500-1700 nm NIR-II window for single cell biomarker profiling in vivo.

Key Features

- A high performance and cost-effective biological imaging system detecting 400-1700 nm fluorescence or luminescence in the visible (400-700 nm) to NIR-I (800-1000 nm) and NIR-II/SWIR (1000-1700 nm) optical windows
- Multiple and customized lasers (808 nm and 975 nm as default)
- For deep tissue, high signal/background ratio *in vivo* small animal imaging, *ex vivo* and *in vitro* organ, tissue and cell culture imaging
- Low and high magnifications for whole mouse-body and single cell/single vessel imaging with switchable lens sets
- Up to 120 frames per second video rate imaging
- Ultra-low noise camera
- Lifetime imaging capability

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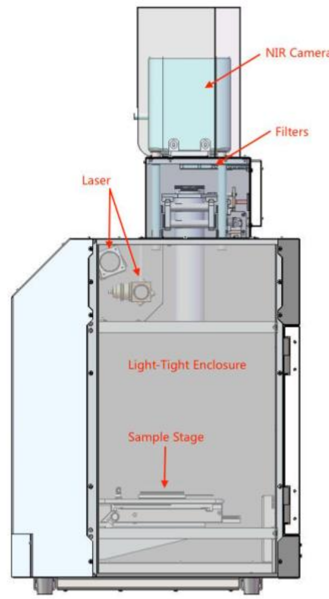
- Multi-color, multiplexed molecular imaging using organic and nanoparticles probes emitting up to 1700 nm

Inside the DeepVision™



- **CCD Camera**
  - 640 x 512 pixels for high imaging resolution
  - 400 nm – 1700 nm with high detection efficiency
  - Camera air cooled to -15 Celsius to ensure low dark current and low noise
  - Lower noise, shorter exposure times (<1/10) than competitor cameras
  - Lifetime imaging mode
  - User friendly software for CW, lifetime and video imaging/recording

- **Imaging Chamber**
  - Light-tight imaging chamber
  - 808 nm and 975 nm lasers
  - Em filter wheels – 3 filters 1000 nm, 1300 nm and 1500 nm long pass.
  - Heating pad for mouse
  - Adjustable imaging field for whole body or high resolution microscopic imaging in vivo
  - Automated X-Y-Z control for mouse stage

DeepVision™ Imaging System

Imaging System Components	Specifications
CCD Size	1 cm x 0.8 cm
Imaging Pixels	640 x 512
Quantum Efficiency	80%-90% in NIR-II 1-1.7 µm; 50-80% in 800-1000 nm
Pixel Size	15 µm x 15 µm
Minimum Field of View (FOV)	1.0 mm x 0.75 mm (microscopy mode)
Maximum Field of View (FOV)	62 mm x 50 mm (whole body mode)
Minimum Image Pixel Resolution	2.34 µm
Stage Temperature	20 °C – 40 °C, heated stage. 37 °C by default.
Power Requirements	110 or 220 V, 50 Hz – 60 Hz
Dark Current	~ 750e^-/p/sec
Read Noise	< 18e^- (High Gain) ~150e^- (Low Gain)
Lasers	808 nm and 975 nm

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Emission Filters	1000 nm long pass, 1300 nm long pass and 1500 nm long pass
CCD Operating Temperature	-15 °C to -30 °C
Imaging System Space Requirements	46*46*110cm (W x D x H)
Lifetime imaging mode	User specified laser excitation time, laser off, followed by specified exposure/imaging time

DeepVision™ uses a new camera technology superior to competitors', allowing for much shorter exposure time and lower background.

High Performance Camera

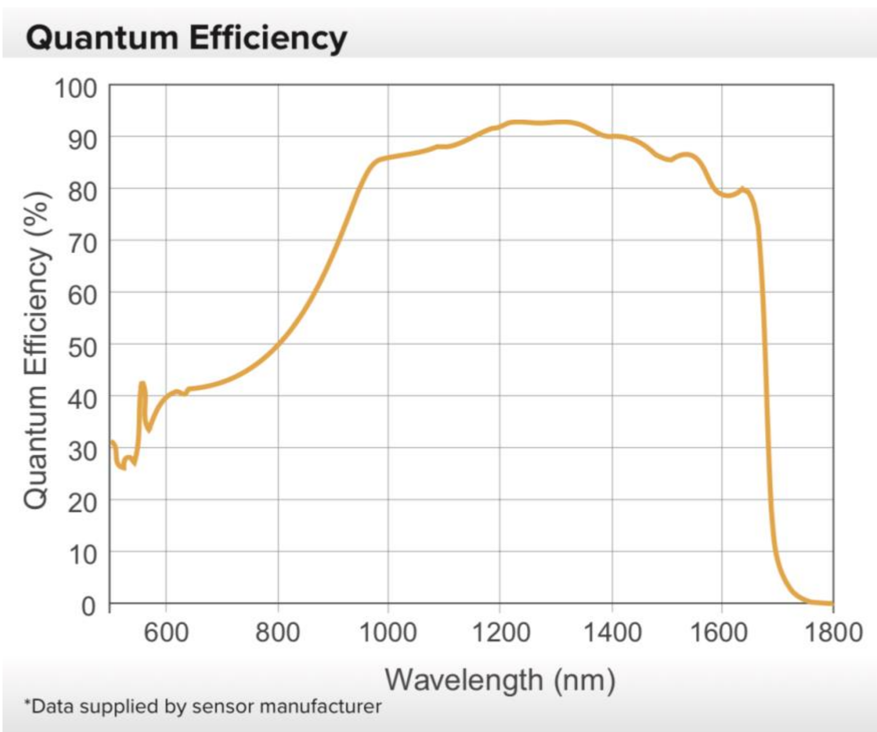

Fig 1. A CCD camera detects in range from visible to NIR-I and NIR-II/SWIR with high quantum efficiency.

Whole Body Imaging

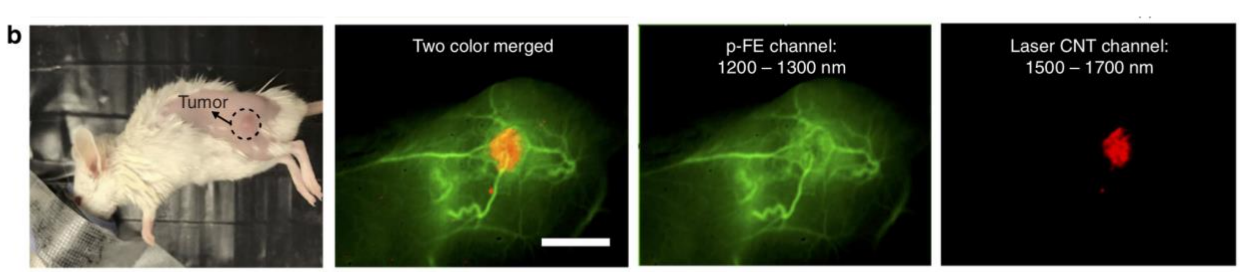

Fig2. A mouse inoculated with a 4T1 tumor was sequentially injected of carbon nanotube (CNTs) and an organic probe p-FE. Utilizing the fluorescence of p-FE emitting in 1100–1300 nm to highlight vasculatures (green) and that of laser-ablation produced CNTs emitting in 1500–1700 nm to highlight the tumor (red), mouse tumor model *in vivo* can be profiled.
Data from Wan et al., *Nature Comm*, 2018

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Imaging of mouse brain through intact scalp and skull

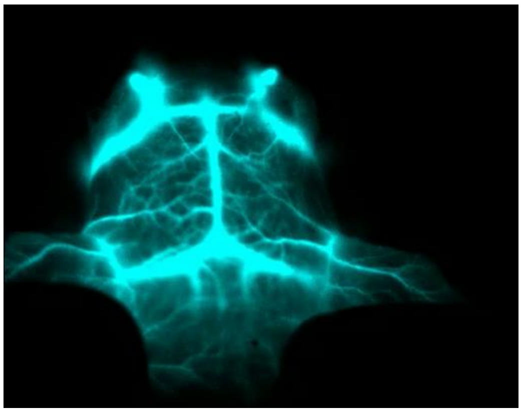

Fig 3. The 1500 – 1700 nm fluorescence signal of rare earth nanoparticles circulating in the cerebral vessels of the mouse was imaged through scalp/skull under a 975 nm laser excitation.
During video recording, the mouse scalp was slightly moved, and the transparency and three-dimensional structure of the mouse head were clearly visible.

The excitation light wavelength is ~980 nm, and the emission light wavelength is ~1600 nm.

In Vivo Two-Plex Molecular Imaging at ~ 1600 nm based on Fluorescence Life Time

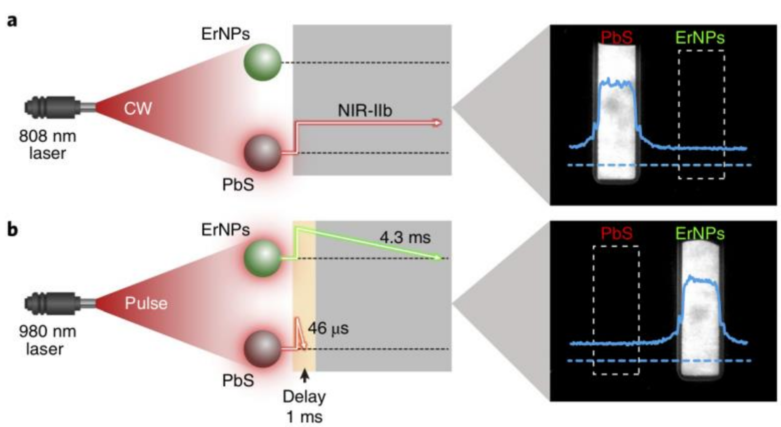

Fig 4. Two-plex molecular imaging of immune response.

Top panel:
The principle of distinguishing short-lived PbS QD fluorescence from long-lived ErNP rare-earth nanoparticle luminescence.

Lower panel:
Two-plex imaging of a CT-26 tumor on a mouse with intravenously injected ErNPs-antiPDL1 (green) and PbS-antiCD8 (red). The zoomed-in high-magnification outlines the tumor with micrometer image resolution.
Modified from Zhong et al., *Nature biotechnology*, 2019

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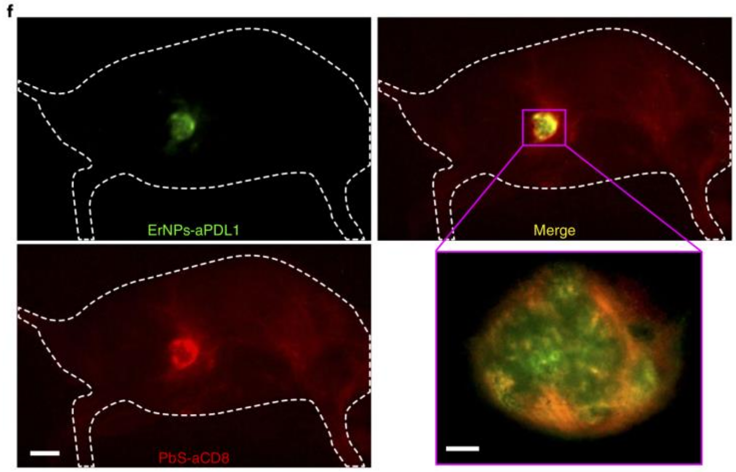

References

Molecular Imaging of Biological Systems With a Clickable Dye in the Broad 800- To 1,700-nm Near-Infrared Window. *PNAS*, 114(5):962-967, 2017.

A Bright Organic NIR-II Nanofluorophore for Three-Dimensional Imaging Into Biological Tissues. *Nat Commun*, 9(1):1171, 2018.

In Vivo Molecular Imaging for Immunotherapy Using Ultra-Bright near-infrared-IIb Rare-Earth Nanoparticles. *Nat Biotechnol*, 37(11):1322-1331, 2019.

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